

## Intake and Performance of Lactating Cows Grazing Diverse Forage Mixtures

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### ABSTRACT

Twenty multiparous Holstein cows in midlactation grazed pastures of 4 forage mixtures in a 12-wk study repeated during 2 grazing seasons to determine if forage mixture complexity affected intake and productivity of lactating dairy cows. The forage mixtures were 1) orchardgrass plus white clover [2 species (SP)]; 2) orchardgrass, white clover, and chicory (3SP); 3) orchardgrass, tall fescue, perennial ryegrass, red clover, birdsfoot trefoil, and chicory (6SP); and 4) 6SP mixture plus white clover, alfalfa, and Kentucky bluegrass (9SP). Total herbage intake was similar among forage mixtures, averaging 12.0 kg/d across all forage mixtures and years. Milk production and composition were not affected by forage mixture or year, and averaged 34.6 kg/d, 3.4%, and 2.8% for milk production, milk fat percentage, and milk protein percentage, respectively. The conjugated linoleic acid content of milk fat was higher for cows that grazed the 3SP, 6SP, and 9SP mixtures than from cows that grazed the 2SP mixture (1.02 vs. 0.87 g of conjugated linoleic acid/100 g of fatty acids, respectively). Blood glucose, blood urea nitrogen, and nonesterified fatty acids were not affected by forage mixture and averaged 69.2 mg/dL, 13.4 mg/dL, and 277.5  $\mu$ Eq/L, respectively. The results of this study indicate that altering the forage mixture in pastures did not affect dry matter intake, milk production, or blood metabolite profiles of lactating cows. The use of complex mixtures of forages in grazing systems should not affect dairy cow performance.

**Key words:** forage mixture, grazing, intake, milk production

### INTRODUCTION

Animal productivity in a grazing system is a function of the output per animal (e.g., milk per cow, weight gain per head) and the number of animals that a unit

of grazing land will support. Voluntary DMI along with stocking rate are key determinants of animal performance on pasture (Fales et al., 1995; Kolver and Muller, 1998). Dry matter intake is strongly affected by multiple factors, including the amount of herbage on offer (Bargo et al., 2002a) as well as its acceptance by the animal (Ganskopp et al., 1997). The amount of herbage grown and consumed is affected by the botanical composition and population of the pastures and the morphology and structure of the sward (Dalley et al., 1999).

Greater plant diversity in grassland plant communities has been linked to increased primary (plant) production (Sanderson et al., 2005), greater stability in response to disturbance (Minns et al., 2001), and reduced weed pressure (Tracy and Sanderson, 2004). With some livestock operations opting for less capital-intensive production systems, emphasis has been placed on low-input pasture systems that rely on complex species mixtures to produce forage. Recent work with forage mixtures in clipped plots showed increased herbage yield in complex vs. simple mixtures of forages (Deak et al., 2004; Tracy and Sanderson, 2004). Although clipped plots provide the opportunity to screen several forage mixtures, the effects of primary (forage) productivity on secondary (animal) productivity are relatively unknown. Very few studies exist in the literature that evaluate the performance of dairy cows with forage mixtures, and nearly all are limited to simple one grass–one legume mixtures, with contradictory results (Wedin et al., 1965; Harris et al., 1997; Phillips and James, 1998; Rutter et al., 2004). Evaluation of more complex forage mixtures on performance of dairy cows has not been evaluated. Therefore, a study was designed to determine the effect of forage mixture complexity in pastures on intake and productivity of grazing lactating dairy cows.

### MATERIALS AND METHODS

#### *Experimental Design, Cows, and Forage Mixtures*

The experiment was conducted under the approval of The Pennsylvania State University Animal Care

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and Use Committee. Twenty multiparous Holstein cows [BW 648 kg  $\pm$  74 kg; milk yield, 46.7 kg  $\pm$  11.5 kg; parity, 3.1  $\pm$  1.1; DIM, 109  $\pm$  21 (mean  $\pm$  SD)] were used in a 12-wk study repeated over 2 grazing seasons beginning on May 5, 2002, and May 6, 2003, respectively. Cows were selected from the herd of The Pennsylvania State University Dairy Cattle Research and Education Center (University Park, PA). Cows were blocked by lactation number and milk yield. These cow blocks were then treated as a unit, remaining together and changing forage mixture as a single unit throughout the experiment. Due to stage of lactation requirements, only 6 cows from the first year were used during the second grazing season.

The forage mixtures compared were 1) 2-species mixture (**2SP**)—orchardgrass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.); 2) 3-species mixture (**3SP**)—orchardgrass, white clover, and chicory (*Cichorium intybus* L.); 3) 6-species mixture (**6SP**)—orchardgrass, tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), red clover (*Trifolium pratense* L.), birdsfoot trefoil (*Lotus corniculatus* L.), and chicory; and 4) 9-species mixture (**9SP**)—6SP mixture plus white clover, alfalfa (*Medicago sativa* L.), and Kentucky bluegrass (*Poa pratensis* L.). Two 1-ha pastures of each forage mixture were established in the fall of 2001. No fertilizer was applied during establishment or for the duration of the trial. Biosecurity policies at The Pennsylvania State University precluded the use of additional dry cows or heifers in rotation with the lactating cows to clean up residual forage. If excessive ungrazed forage remained after grazing, pastures were clipped to maintain a similar vegetative state in each paddock and the clipped residue was left in place. Mechanical harvest or clipping of excess forage is a common practice on grazing dairies in the northeastern United States to maintain the pasture in a vegetative state and control weeds.

The experimental design was a randomized complete block. Cow blocks (5 cows/block) were assigned to 1 of 4 forage mixtures for each 3-wk period. Cow blocks switched forage mixtures every 3 wk, so by the end of the experiment, all cow blocks had grazed all 4 forage mixtures.

Two weeks before the start of the experiment (mid-April of each year), cows were adapted to pasture. Cows had access to the experimental pastures (for exposure to all forage species) for 2 h during the first adaptation day. This was increased by 1 to 2 h each day until the cows had 24-h access to pasture before the beginning of the experimental periods. Cows were individually fed a corn-based concentrate at a rate of 1 kg of DM/4 kg of milk (based on pretrial milk yield)

**Table 1.** Ingredient and chemical composition (mean  $\pm$  SD) of the concentrate mixture (DM basis)

Item	
Ingredient composition, %	
Dry ground corn	41.7
Wheat middlings	18.8
Soyhulls	16.7
Corn distillers (dark)	8.6
Citrus pulp	5.2
Heat-treated soybean meal	3.1
Soybean meal (48%)	2.9
Plain salt	0.90
Magnesium oxide	0.73
Calcium carbonate	0.61
Dicalcium phosphate	0.34
Agmate (KMS) <sup>1</sup>	0.20
Selenium premix (0.06%)	0.11
Trace mineral premix <sup>2</sup>	0.06
Vitamins A, D, E	0.05
Chemical composition <sup>3</sup>	
DM, %	95.2 $\pm$ 1.2
OM, % of DM	90.3 $\pm$ 0.9
CP, % of DM	14.1 $\pm$ 0.6
Soluble CP, % of CP	15.9 $\pm$ 0.7
TNC, <sup>4</sup> % of DM	41.8 $\pm$ 1.8
NDF, % of DM	28.9 $\pm$ 1.1
ADF, % of DM	17.4 $\pm$ 0.8
IVDMD, <sup>5</sup> % of DM	74.0 $\pm$ 3.8

<sup>1</sup>99% ash, 11.2% Mg, 18.4% K, and 22.4% S.

<sup>2</sup>98.6% ash; 0.58% Ca; 1,390 mg/kg Co; 41,649 mg/kg Cu; 2,780 mg/kg I; 10,412 mg/kg Fe; 124,947 mg/kg Mn; 16.1% S, and 124,947 mg/kg Zn.

<sup>3</sup>Averaged over the 2 yr of study (2002 and 2003).

<sup>4</sup>TNC = Total nonstructural carbohydrates.

<sup>5</sup>IVDMD = In vitro DM disappearance.

split in 2 equal feedings after milking (Table 1). An upper limit of 9.2 kg of DM/d per cow was established to minimize the risk of metabolic problems in the rumen. Any concentrateorts were removed and weighed after each feeding. The total diet consisted of 40% concentrate and 60% forage in 2002, and 47% concentrate and 53% forage in 2003, with 100% of the forage coming from pasture both years.

Measurement of pregrazing herbage mass and botanical composition were described previously (Sanderson et al., 2005). In brief, 30 readings were taken in each pasture with a calibrated rising-plate meter (Jenn Quip model, Feilding, New Zealand) twice each week. A single calibration equation was developed for all forage mixtures within each year. The calibration equation for 2002 was herbage mass (kg/ha of DM) = 353 + 84.5  $\times$  (rising plate reading),  $r^2 = 0.82$ , root error mean square = 318 kg/ha of DM,  $n = 78$ . The equation for 2003 was herbage mass = -30 + 90.6  $\times$  (rising plate reading),  $r^2 = 0.85$ , root error mean square = 295 kg/ha of DM,  $n = 80$ . Botanical composition was measured during 2 consecutive weeks before the start of the first intake period and during 2 consecutive weeks of each

of the 4 intake periods. At each sampling, herbage in ten 0.03-m<sup>2</sup> quadrats was hand-clipped to a 1-cm stubble height in each pasture and bulked. The bulked herbage was hand separated into dead material, sown forage species, and weeds (unsown species), dried at 55°C for 48 h, and weighed. Data are averages across all sampling weeks for each grazing season. Pregrazing herbage mass was used to adjust paddock size based on herbage yield. Cows were allotted 25 kg/d of DM per cow of herbage mass. Using temporary polywire, new paddocks were constructed daily. Daily paddocks were subdivided and back-fenced so that cows were offered fresh pasture (1/2 of the daily herbage allowance) after each milking. During slower periods of pasture growth, pastures were rested for 7 d in mid-June each year (between periods 2 and 3) before resuming the trial. During this pasture recovery period, cows were kept on pastures that contained the same forages as the experimental pastures.

Cows were milked at 0500 and 1700 h and received bST injections every 2 wk. Walking distance from the pasture to the milking parlor averaged 0.9 km (range: 0.75 to 1.2 km); therefore, cows walked an average of 3.6 km/d.

### Experimental Measures and Sample Analyses

Total DMI was estimated using Cr<sub>2</sub>O<sub>3</sub> as an indigestible fecal marker during wk 3 of each of the 4 experimental periods (Holden et al., 1994). Beginning on d 8 of each period and continuing for 11 d, Cr<sub>2</sub>O<sub>3</sub> was administered twice daily (10 g/d) after each milking (0600 and 1800 h) via gelatin capsules. Fecal grab samples were collected at 0600 and 1800 h on d 15 to 19 of each period and immediately frozen (−20°C).

On d 14 to 18 of each period, samples of concentrate were collected for nutritional analyses. Pasture samples were plucked by hand to approximate the height at which the cows grazed to be used to determine forage quality during the intake period. Hand-plucked pasture samples were also taken once weekly throughout the grazing season to monitor forage quality. Concentrate samples were taken once weekly. Samples were dried at 55°C in a forced air oven for 48 h and ground through a 1-mm screen (Wiley mill, Thomas Scientific, Philadelphia, PA). Weekly concentrate and daily hand-plucked pasture samples (intake period) were composited by period. Weekly hand-plucked pasture samples were kept as weekly samples. Concentrate and pasture samples were analyzed for DM, CP, ash (AOAC, 1990), soluble CP (Roe et al., 1990), ADF, and NDF (ANKOM<sup>200</sup> Fiber Analyzer, Ankom Technology Corp., Fairport, NY), nonstructural carbohydrates (NSC; Smith, 1981, modified to use potassium ferricy-

anide as the colorimetric indicator), and in vitro DM disappearance (IVDMD) by a 2-stage procedure (Tilley and Terry, 1963). Concentrate and pasture samples were analyzed for mineral content by wet chemistry (Dairy One Forage Analysis Laboratory, Ithaca, NY).

Fecal samples were thawed, dried at 55°C in a forced air oven for 96 h, and ground through a 1-mm screen (Wiley mill). A composited sample per cow was made for each period (by year) for fecal output estimates. Fecal samples were analyzed for CP (AOAC, 1990), NDF (Ankom<sup>200</sup> Fiber Analyzer, Ankom Technology Corp.), and Cr (Parker et al., 1989).

Intake was estimated using the equation  $DMI = \text{fecal output} / (1 - IVDMD)$ . Fecal output was estimated using the equation  $\text{fecal output} = (g \text{ of Cr dosed per d}) / (g \text{ of Cr/g of fecal DM})$ . Pasture DMI was estimated by difference between the estimated total DMI (based on fecal output) and the known concentrate DMI. The proportionate IVDMD (using known concentrate DMI and estimated pasture DMI) of the pasture and the concentrate were used to determine total DMI (Holden et al., 1994).

Milk production was recorded daily. Milk samples were collected twice weekly during wk 2 and 3 of each period and preserved with 2-bromo-2-nitropropane-1,3 diol. Milk fat and protein, lactose, and MUN were analyzed by infrared spectrophotometry (Foss 605B MilkoScan, Foss Electric, Hillerød, Denmark; AOAC, 1990) by the Pennsylvania DHIA laboratory. Milk fatty acids (FA) were extracted and subsequently transmethylated as described by Baumgard et al. (2002). Fatty acid methyl esters were quantified by gas chromatography (Hewlett Packard 6890, Foster City, CA). Separations were made with the SP 2560 fused silica capillary column (Supelco, Bellefonte, PA). The column was 100 m in length, with an inner diameter of 0.25 mm and a film thickness of 0.2 µm. Oven temperatures were initially set at 80°C and held for 15 min. Helium was the carrier gas and flowed at 1.1 mL/min (17 cm/s, velocity). Airflow was set at 400 mL/min and the makeup gas, hydrogen, was 45 mL/min. Inlet and detector temperatures were set at 250°C. Retention times were determined with pure methyl ester standards (Nu-Chek Prep, Elysian, MN; GLC-60, *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and *trans*-10, *cis*-12 CLA). A butter oil reference standard (CRM 164; Commission of the European Community Bureau of References, Brussels, Belgium) was used to determine the efficiencies of recoveries and correction factors for individual FA as described by Baumgard et al. (2002). Molar basis FA production (mmol/d) was estimated by dividing the yield (on a mass basis) by the molecular weight of each individual FA as described by Peterson et al. (2002). Additionally, milk samples were



collected approximately 2 wk before and 2 wk after the experiment (all cows were consuming the same TMR in confinement during both periods) and analyzed for milk FA content for comparison.

Twice weekly, at 0600 h, during wk 2 and 3 of each period before cows received concentrate, blood samples were collected from the coccygeal vein into one 20-mL evacuated tube containing sodium heparin, and one 10-mL evacuated tube containing potassium oxylate-sodium fluoride (glycolytic inhibitor). Blood was immediately placed on ice and transported to the laboratory. Samples were centrifuged at  $3,000 \times g$  for 15 min at 4°C. Heparinized plasma was analyzed for urea N (Stanbio Urea Nitrogen kit 580, Stanbio Laboratory, Inc., San Antonio, TX) and NEFA (NEFA C-kit no. 990-75401, Wako Chemicals USA, Inc., Richmond, VA) concentrations. Nonheparinized plasma was analyzed for glucose (Glucose kit no. 510, Sigma Chemical Co., St. Louis, MO) concentration.

Urine samples were taken by vulval stimulation twice daily after each milking on 2 consecutive days in wk 3 of each period. Samples were acidified with HCl to maintain a pH below 2 and stored at -20°C. Urine samples were later thawed, composited to one sample per period per cow, and analyzed for allantoin (Chen, 1989) and creatinine (Sigma kit no. 555-a) to estimate microbial protein synthesis in the rumen (Gonda, 1995).

### Statistical Analyses

Animal performance data were analyzed using the PROC MIXED procedure of SAS (Littell et al., 1996). The model included the fixed effects of treatment (forage mixture), week, period, year, cow block, treatment  $\times$  year, and treatment  $\times$  period interactions, the random effect of cow nested within treatment (except pasture analyses), and the residual error. For each animal variable analyzed, cow nested within treatment was subjected to 3 covariance structures in PROC MIXED: unstructured, compound symmetry, and autoregressive order 1 covariance. The covariance structure that resulted in the smallest Akaike's information criterion and Schwarz Bayesian criterion was used.

Forage yield data were analyzed as a randomized complete block design with the PROC MIXED procedure of SAS mixed models procedure in SAS (Littell et al., 1996). Treatments were considered fixed effects and blocks were random. Years were analyzed separately.

Least squares means and SEM are reported for all data. Nonsignificant interactions were not reported. When significant ( $P < 0.05$ ) effects due to dietary treat-

ment, period, year, or any interaction were detected, mean separation was conducted by the PDIF option in SAS (SAS Institute, 1999).

## RESULTS

### Pasture Management and Forage Quality

Pasture forage quality averaged 23.0% CP, 18.6% total NSC, 22.1% ADF, 32.3% NDF, and 64.1% IVDMD across forage mixtures and years (Table 2). The average pregrazing botanical composition of the 2SP mixture remained about the same in both years with 32 to 38% legume, 43 to 49% orchardgrass, and 21 to 13% weed from 2002 to 2003 (Sanderson et al., 2005; Table 3). The 3SP mixture changed from 25 to 52% legume, 18 to 25% grass, 34 to 16% chicory, and 23 to 6% weed for 2002 and 2003, respectively. The 6SP mixture changed from 28 to 24% legume, 23 to 42% grass, 35 to 25% chicory, and 10% weed for 2002 and 2003, respectively. The 9SP mixture changed from 44 to 65% legume, 12% grass, 39 to 19% chicory, and 4% weed for 2002 and 2003, respectively. Chicory decreased in each mixture, whereas the orchardgrass and legume proportions increased from 2002 to 2003.

### DM and Nutrient Intake

Dry matter intake was not affected ( $P > 0.05$ ) by forage mixture; however, there was a significant ( $P < 0.05$ ) year effect for pasture DMI and total DMI expressed as kilograms per day and as a percentage of BW (Table 4). Pasture DMI for all forage mixtures was slightly higher during 2002 than in 2003.

### Milk Production and Composition

Milk production and 4% FCM were not affected ( $P > 0.05$ ) by forage mixture or by year (Table 5). Forage mixture did not affect ( $P > 0.05$ ) 4% FCM, milk fat percentage, milk protein percentage, MUN, or lactose (Table 5). There was a significant period effect ( $P < 0.05$ ) for FCM, milk fat yield, and MUN.

### Milk Fatty Acids

The FA composition of milk for the pretrial, experimental, and posttrial periods is shown in Table 6. Short- and medium-chain fatty acids were not affected by forage mixture during the experimental period ( $P > 0.05$ ). Milk from cows grazing the 2SP pasture mixture had lower ( $P < 0.05$ ) C18:2 and CLA content than the

**Table 2.** Chemical composition of the hand-plucked pasture samples for 4 forage mixtures (averaged across all periods; DM basis)

	Forage mixture <sup>1</sup>				Mean	SEM
	2SP	3SP	6SP	9SP		
DM, %						
2002	18.4 <sup>a</sup>	17.2 <sup>ab</sup>	17.0 <sup>ab</sup>	16.0 <sup>b</sup>	17.2	1.0
2003	19.5	17.9	18.1	18.7	18.6	1.0
OM, % of DM						
2002	90.4 <sup>a</sup>	89.5 <sup>b</sup>	89.4 <sup>b</sup>	89.2 <sup>b</sup>	89.6	0.2
2003	89.9 <sup>a</sup>	89.0 <sup>b</sup>	89.1 <sup>b</sup>	88.8 <sup>b</sup>	89.2	0.2
CP, % of DM						
2002	21.8 <sup>a</sup>	20.0 <sup>b</sup>	21.2 <sup>ab</sup>	22.5 <sup>a</sup>	21.4	0.5
2003	23.2 <sup>b</sup>	24.6 <sup>ab</sup>	24.1 <sup>b</sup>	25.9 <sup>a</sup>	24.5	0.5
Soluble CP, % of DM						
2002	6.1	6.8	6.6	6.1	6.4	0.4
2003	7.5 <sup>bc</sup>	8.0 <sup>ab</sup>	7.1 <sup>c</sup>	8.5 <sup>a</sup>	7.8	0.4
TNC, <sup>2</sup> % of DM						
2002	18.4 <sup>b</sup>	20.9 <sup>a</sup>	21.5 <sup>a</sup>	21.7 <sup>a</sup>	20.6	0.7
2003	15.2 <sup>b</sup>	16.9 <sup>ab</sup>	18.3 <sup>a</sup>	16.2 <sup>b</sup>	16.7	0.7
NDF, % of DM						
2002	36.6 <sup>a</sup>	31.6 <sup>b</sup>	29.3 <sup>b</sup>	24.5 <sup>c</sup>	30.5	1.5
2003	40.8 <sup>a</sup>	31.8 <sup>b</sup>	35.4 <sup>b</sup>	28.2 <sup>b</sup>	34.1	1.5
ADF, % of DM						
2002	23.2 <sup>a</sup>	22.0 <sup>a</sup>	20.5 <sup>ac</sup>	18.8 <sup>bc</sup>	21.1	0.8
2003	25.2 <sup>a</sup>	22.7 <sup>b</sup>	22.8 <sup>b</sup>	21.8 <sup>b</sup>	23.1	0.8
IVDMD, <sup>3</sup> % of DM						
2002	66.6 <sup>c</sup>	70.4 <sup>ab</sup>	67.2 <sup>bc</sup>	70.9 <sup>a</sup>	68.8	1.2
2003	58.4 <sup>bc</sup>	60.6 <sup>ab</sup>	57.2 <sup>c</sup>	61.0 <sup>a</sup>	59.3	1.2
Ca, % of DM						
2002	1.00 <sup>b</sup>	1.25 <sup>b</sup>	1.37 <sup>a</sup>	1.38 <sup>a</sup>	1.25	0.03
2003	0.94 <sup>d</sup>	1.30 <sup>b</sup>	1.12 <sup>c</sup>	1.44 <sup>a</sup>	1.20	0.03
P, % of DM						
2002	0.42 <sup>c</sup>	0.54 <sup>a</sup>	0.50 <sup>b</sup>	0.49 <sup>b</sup>	0.49	0.01
2003	0.47	0.49	0.46	0.49	0.48	0.01
Mg, % of DM						
2002	0.25 <sup>b</sup>	0.27 <sup>b</sup>	0.31 <sup>ab</sup>	0.28 <sup>ab</sup>	0.28	0.01
2003	0.26 <sup>b</sup>	0.28 <sup>ab</sup>	0.30 <sup>a</sup>	0.29 <sup>ab</sup>	0.28	0.01
K, % of DM						
2002	3.17 <sup>c</sup>	3.83 <sup>a</sup>	3.65 <sup>b</sup>	3.68 <sup>b</sup>	3.58	0.04
2003	3.40 <sup>c</sup>	3.54 <sup>b</sup>	3.60 <sup>b</sup>	3.70 <sup>a</sup>	3.56	0.04
Na, % of DM						
2002	0.020 <sup>c</sup>	0.036 <sup>b</sup>	0.054 <sup>a</sup>	0.040 <sup>b</sup>	0.038	0.002
2003	0.015 <sup>c</sup>	0.035 <sup>a</sup>	0.039 <sup>a</sup>	0.028 <sup>b</sup>	0.029	0.002
S, % of DM						
2002	0.30 <sup>c</sup>	0.35 <sup>b</sup>	0.36 <sup>b</sup>	0.41 <sup>a</sup>	0.36	0.01
2003	0.30 <sup>ab</sup>	0.31 <sup>ab</sup>	0.33 <sup>a</sup>	0.29 <sup>b</sup>	0.31	0.01

<sup>a-d</sup>Means within the same row (within year) with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>2SP = Orchardgrass and white clover; 3SP = orchardgrass, white clover, and chicory; 6SP = orchardgrass, tall fescue, perennial ryegrass, red clover, birdsfoot trefoil, and chicory; 9SP = 6SP mixture plus white clover, alfalfa, and Kentucky bluegrass.

<sup>2</sup>TNC = Total nonstructural carbohydrates.

<sup>3</sup>IVDMD = In vitro DM disappearance.

more complex mixtures. No other long-chain fatty acids were affected by forage mixture ( $P > 0.05$ ). The CLA and C18:3 content were lower ( $P < 0.05$ ) during the pre- and posttrial periods than during the experimental periods.

### Plasma and Urine Metabolites

Plasma glucose, plasma urea nitrogen, and NEFA concentrations were not affected ( $P > 0.05$ ) by forage

mixture (Table 7). Plasma glucose concentration was not affected ( $P > 0.05$ ) by year; however, year had a significant effect ( $P < 0.05$ ) on plasma urea nitrogen and NEFA concentrations. Urinary allantoin concentrations were lower ( $P < 0.05$ ) in the 9SP mixture in 2002, and higher ( $P < 0.05$ ) in the 2SP mixture in 2003 than the other mixtures. Creatinine concentrations were higher ( $P < 0.05$ ) in the 2SP mixture only in 2003. The allantoin/creatinine ratio was not affected ( $P > 0.05$ ) by forage mixture or year.

**Table 3.** Pregrazing botanical composition of 4 forage mixtures over 2 grazing seasons (2002 and 2003; mean for the grazing season within each year)

Forage mixture <sup>1</sup>	White clover	Orchard-grass	Chicory	Red clover	Tall fescue	Birdsfoot trefoil	Alfalfa	Blue-grass	Perennial ryegrass	Weeds
Percentage of green DM										
2002										
2SP	32.0	42.6	—	—	—	—	—	—	—	21.4
3SP	25.3	17.5	33.6	—	—	—	—	—	—	22.9
6SP	—	18.2	34.6	25.8	4.8	2.0	—	0.2	—	11.5
9SP	20.1	4.2	39.1	19.5	3.3	0.5	3.8	0.5	4.0	4.9
2003										
2SP	38.3	48.9	—	—	—	—	—	—	—	12.8
3SP	51.9	25.3	16.5	—	—	—	—	—	—	6.4
6SP	—	37.6	24.8	21.8	4.0	2.0	—	0.1	—	9.7
9SP	53.5	10.1	18.9	9.1	1.3	0.6	1.6	0.2	1.8	2.9

<sup>1</sup>2SP = Orchardgrass and white clover; 3SP = orchardgrass, white clover, and chicory; 6SP = orchardgrass, tall fescue, perennial ryegrass, red clover, birdsfoot trefoil, and chicory; 9SP = 6SP mixture plus white clover, alfalfa, and Kentucky bluegrass.

## DISCUSSION

### Pasture Management and Forage Quality

Forage quality was within the range summarized by Muller and Fales (1998) for cool-season forages in Pennsylvania. The IVDMD was higher than that reported by others (Kolver et al., 1998; Bargo et al., 2002b). Barry (1998) reported that chicory had a higher apparent OM digestibility (82%) when compared with perennial ryegrass (74%). The NSC content and mineral content were generally higher for the 3SP, 6SP, and 9SP mixtures than the 2SP mixture, probably due to the presence of chicory. Chicory has a greater

mineral and NSC content than do cool-season grasses (Barry, 1998).

Total seasonal (April to October) herbage yields were 4,800, 7,400, 7,900, and 7,500 kg of DM/ha in 2002 for the 2SP, 3SP, 6SP, and 9SP mixtures, respectively (Sanderson et al., 2005). The 2SP mixture yielded less than the 3SP, 6SP, and 9SP mixtures, which did not differ from each other in yield. Total seasonal herbage yields in 2003 averaged 9,900 kg of DM/ha with no differences among mixtures (Sanderson et al., 2005). Herbage yield was lower in 2002 than in 2003 due to lower rainfall (46% below average) and higher temperatures (1°C above average) in the

**Table 4.** Dry matter and nutrient intake of dairy cows grazing 4 forage mixtures over 2 grazing seasons (2002 and 2003)

	Forage mixture <sup>1</sup>				SEM	P-value
	2SP	3SP	6SP	9SP		
Pasture DMI, <sup>2,3</sup> kg/d						
2002	13.7	13.7	13.6	12.9	0.40	0.25
2003	11.1	10.5	10.5	10.2	0.40	0.25
Supplement DMI, kg/d						
2002	9.2	9.2	9.2	9.2	<0.1	—
2003	9.2	9.2	9.2	9.2	<0.1	—
Total DMI, kg/d <sup>3</sup>						
2002	22.9	22.9	22.8	22.1	0.5	0.36
2003	20.3	19.7	19.7	19.4	0.5	0.36
Total DMI, % of BW <sup>4</sup>						
2002	3.68	3.61	3.63	3.51	0.11	0.27
2003	3.20	3.08	3.10	3.04	0.11	0.27

<sup>a,b</sup>Means within the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>2SP = Orchardgrass and white clover; 3SP = orchardgrass, white clover, and chicory; 6SP = orchardgrass, tall fescue, perennial ryegrass, red clover, birdsfoot trefoil, and chicory; 9SP = 6SP mixture plus white clover, alfalfa, and Kentucky bluegrass.

<sup>2</sup>Estimated using Cr<sub>2</sub>O<sub>3</sub>.

<sup>3</sup>Significant year effect ( $P < 0.05$ ).

<sup>4</sup>BW: 648 ± 74 kg.

**Table 5.** Milk yield and composition of dairy cows grazing 4 forage mixtures over 2 grazing seasons (2002 and 2003; averaged across the 2 yr unless otherwise noted)

	Forage mixture <sup>1</sup>				SEM	P-value
	2SP	3SP	6SP	9SP		
Milk, kg/d	33.9	35.4	34.4	34.3	1.3	0.87
Milk fat, %	3.5	3.4	3.5	3.3	0.12	0.53
4% FCM, kg/d	30.1	31.1	30.7	30.1	1.6	0.91
True protein, %	2.80	2.82	2.79	2.81	0.04	0.84
Lactose, %	4.63	4.63	4.62	4.63	0.09	0.99
MUN, mg/dL <sup>2</sup>						
2002	12.4	11.1	12.3	12.5	0.3	0.08
2003	14.5	15.2	13.4	15.0	0.3	0.08

<sup>1</sup>2SP = Orchardgrass and white clover; 3SP = orchardgrass, white clover, and chicory; 6SP = orchardgrass, tall fescue, perennial ryegrass, red clover, birdsfoot trefoil, and chicory; 9SP = 6SP mixture plus white clover, alfalfa, and Kentucky bluegrass.

<sup>2</sup>Significant year effect ( $P < 0.05$ ).

summer of 2002 compared with 2003, which had 59% above-average rainfall and 2.5°C below average summer temperatures.

The proportion of chicory in the herbage of the 3SP, 6SP, and 9SP mixtures was higher in the postgrazing residue than in the pregrazing herbage (Sanderson et al., 2005). This suggests that cattle preferred the grasses and legumes and partly avoided the chicory. Chicory contains sesquiterpene lactones, which impart a bitter taste to the herbage and may deter animal grazing (Foster et al., 2002). There may have also been

selective grazing of the chicory, as the grazing season progressed and the chicory began to bolt, the cattle appeared to favor the young leaf growth of the chicory while avoiding the mature leaves and bolting stem, which would have been left in the postgrazing samples.

### DM and Nutrient Intake

Dry matter intake was slightly lower during 2003 than in 2002, partly a result of higher NDF and lower IVDMD in 2003. Variation associated with the Cr<sub>2</sub>O<sub>3</sub>

**Table 6.** Fatty acid (FA) profile in milk fat of dairy cows grazing 4 forage mixtures over 2 grazing seasons (2002 and 2003)

FA, g/100 g of FA	Pretrial period	Forage mixture <sup>2</sup>				SEM	Posttrial period
	Mean ( $\pm$ SD) <sup>1</sup>	2SP	3SP	6SP	9SP		Mean ( $\pm$ SD) <sup>3</sup>
C4:0	3.11 $\pm$ 0.72	2.75	2.83	2.92	2.82	0.21	3.95 $\pm$ 1.99
C6:0	2.41 $\pm$ 0.44	2.20	2.14	2.22	2.09	0.10	2.76 $\pm$ 1.33
C8:0	1.36 $\pm$ 0.23	1.25	1.24	1.22	1.21	0.05	1.44 $\pm$ 0.43
C10:0	2.83 $\pm$ 0.61	2.45	2.49	2.44	2.44	0.08	2.68 $\pm$ 0.52
C12:0	2.96 $\pm$ 0.65	2.55	2.53	2.44	2.51	0.07	3.10 $\pm$ 0.54
Total short chain	12.67 $\pm$ 2.42	11.06	11.22	11.21	11.08	0.39	13.72 $\pm$ 3.59
C14:0	9.32 $\pm$ 1.56	9.11	9.10	9.07	8.85	0.16	9.87 $\pm$ 1.42
C14:1	0.68 $\pm$ 0.19	0.84	0.84	0.82	0.80	0.03	0.89 $\pm$ 0.21
C16:0	23.27 $\pm$ 2.03	24.75	25.35	24.61	24.41	0.53	29.20 $\pm$ 4.49
C16:1	1.20 $\pm$ 0.37	1.33	1.40	1.34	1.37	0.05	1.58 $\pm$ 0.54
Total medium chain	35.17 $\pm$ 2.99	36.95	37.98	36.72	36.29	0.62	43.14 $\pm$ 3.96
C18:0	15.14 $\pm$ 1.72	14.13	13.67	14.29	13.04	0.35	11.57 $\pm$ 2.74
C18:1	28.89 $\pm$ 3.53	30.84	30.80	30.89	31.39	0.72	24.34 $\pm$ 5.08
C18:2	4.55 $\pm$ 0.43	4.06 <sup>c</sup>	4.69 <sup>ab</sup>	4.50 <sup>b</sup>	4.94 <sup>a</sup>	0.12	3.74 $\pm$ 0.90
CLA, <i>cis</i> -9, <i>trans</i> -11	0.52 $\pm$ 0.09*	0.87 <sup>b</sup>	1.02 <sup>a</sup>	0.99 <sup>a</sup>	1.04 <sup>a</sup>	0.04	0.46 $\pm$ 0.13*
C18:3	0.44 $\pm$ 0.12*	1.06	0.99	1.03	0.94	0.05	0.64 $\pm$ 0.16*
Total long chain	50.08 $\pm$ 4.24	51.64	51.88	52.35	52.01	0.76	41.60 $\pm$ 6.32
Saturated FA, % of total FA	62 $\pm$ 3.71	61	61	61	59	1.26	67 $\pm$ 4.50

<sup>1</sup>Pretrial samples were collected on April 15 (2002 and 2003) while cows were consuming a TMR (before transition to pasture).

<sup>2</sup>Samples were collected twice weekly from May 6 through August 3, 2002 (yr 1), and May 5 through August 2, 2003 (yr 2).

<sup>3</sup>Posttrial samples were collected on August 19 (2002 and 2003), 16 and 17 d (respectively) after cows were removed from pasture and placed back on a TMR.

\*Significantly different from experimental periods ( $P < 0.05$ ).

**Table 7.** Blood and urine metabolites of dairy cows grazing 4 forage mixtures over 2 grazing seasons (2002 and 2003)

	Forage mixture <sup>1</sup>				SEM	P-value
	2SP	3SP	6SP	9SP		
Blood						
Glucose, mg/dL						
2002	70.3	66.1	66.6	66.7	4.6	0.49
2003	68.3	69.7	69.4	71.3	4.8	0.54
PUN, <sup>2</sup> mg/dL						
2002	14.5	13.1	13.7	14.0	0.5	0.13
2003	12.8	13.1	12.0	13.9	0.5	0.19
NEFA, <sup>3</sup> μEq/L						
2002	228.8	256.1	256.0	266.8	19.5	0.87
2003	320.5	282.4	299.0	309.7	19.3	0.89
Urine						
Allantoin (A), mg/L						
2002	1,501.4 <sup>a</sup>	1,429.7 <sup>a</sup>	1,425.9 <sup>a</sup>	1,293.2 <sup>b</sup>	82.9	<0.001
2003	1,659.7 <sup>a</sup>	1,455.7 <sup>b</sup>	1,443.8 <sup>b</sup>	1,394.1 <sup>b</sup>	83.9	<0.001
Creatinine (C), mg/L						
2002	470.3	430.0	432.1	454.9	29.5	0.25
2003	547.4 <sup>a</sup>	432.5 <sup>b</sup>	460.0 <sup>b</sup>	440.8 <sup>b</sup>	29.5	<0.001
A/C ratio						
2002	3.21	3.38	3.37	2.99	0.15	0.21
2003	3.13	3.37	3.19	3.24	0.15	0.22

<sup>a,b</sup>Means within the same row (within year) with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>2SP = Orchardgrass and white clover; 3SP = orchardgrass, white clover, and chicory; 6SP = orchardgrass, tall fescue, perennial ryegrass, red clover, birdsfoot trefoil, and chicory; 9SP = 6SP mixture plus white clover, alfalfa, and Kentucky bluegrass.

<sup>2</sup>PUN = Plasma urea nitrogen; significant year effect ( $P < 0.05$ ).

<sup>3</sup>Significant year effect ( $P < 0.05$ ).

technique and use of different cows also contributed to year differences. Our pasture DMI values, however, were similar to those of others under similar experimental conditions (Holden et al., 1994; Bargo et al., 2002b). There was no effect of pasture species mixture on DMI of lactating dairy cows.

There have been few comparisons of herbage intake and milk production from forage mixtures on pasture, particularly in studies conducted in the United States. Others in Europe and New Zealand have reported improved herbage intake and milk production on grass-legume swards compared with grass monocultures (Harris et al., 1997; Phillips and James, 1998). Contrarily, Wedin et al. (1965) reported that complex forage mixtures supported less milk production than a simple grass-legume mixture or N-fertilized grass. In our study, increasing mixture complexity by combining chicory and several species of grasses and legumes did not affect herbage intake or milk production. In addition, cows received 40% of their diet from a concentrate supplement, an economically beneficial practice due to milk to feed price ratios (Soder and Rotz, 2001). Supplementation may affect grazing behavior (Rook et al., 1994; Soriano et al., 2000), perhaps resulting in changes in grazing selection. Research is needed to determine if responses are the same for unsupple-

mented vs. supplemented cows (or type of supplementation) for full lactation cycles.

### Milk Fatty Acids

During the experimental grazing periods, milk CLA content was 188 and 213% higher than in the pre- and posttrial periods respectively (when cows were fed a TMR). Other studies showed that CLA content of milk increased between 150 to 500% when pasture was the primary source of forage in the diet of lactating dairy cows (Kelly et al., 1998; Dhiman et al., 1999; Schroeder et al., 2003). The increased CLA content in milk from cows grazing the 3SP, 6SP, and 9SP forage mixtures may have been a result of increased unsaturated FA content of the chicory (8 to 10 mg of linoleic acid/g of DM, 30 to 40 mg of  $\alpha$ -linolenic acid/g of DM; W. Clapham, USDA-ARS, Beaver, WV; personal communication). Conversely, if the cows avoided grazing chicory, they may have consumed more legumes, which contain higher linoleic acid levels than cool-season grasses (Engelhart, 2003).

### Plasma and Urine Metabolites

Plasma glucose, plasma urea nitrogen, and NEFA concentrations are within the ranges reported in other



grazing studies using cows from the same research herd (Kolver and Muller, 1998; Bargo et al., 2002b). Other studies (Carruthers and Neil, 1997; Bargo et al., 2002b) reported similar values for allantoin concentrations (1,992 and 1,696 mg/L, respectively) for grazing dairy cows supplemented with concentrate. The allantoin/creatinine ratio, an indirect indicator of ruminal microbial protein synthesis (Gonda, 1995) was not affected by species mixture or by weather conditions, suggesting that ruminal microbial protein synthesis was not altered by forage species mixture.

## CONCLUSIONS

Forage mixture did not affect DMI, milk production, milk composition, or blood metabolites of high-producing cows. Although individual cow performance was not affected, our previous research demonstrated an herbage yield benefit for complex mixtures during a dry year and reduced weed invasion during establishment that may allow for increased stocking rates and greater milk production per hectare.

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